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Elevated Ferritin Is Associated with Relapse after Autologous Hematopoietic Stem Cell Transplantation for Lymphoma

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Elevated serum ferritin is associated with reduced survival following allogeneic transplantation and an increased risk of toxic and infectious complications after autologous hematopoietic stem cell transplantation (ASCT). We studied 315 patients who underwent ASCT for Hodgkin (HL) or non-Hodgkin lymphoma (NHL) at our institution in whom pretransplantation ferritin was available to determine its association with survival. On multivariate analysis, a pretransplantation ferritin >685 ng/mL was associated with significantly lower overall (OS; $P = .002$) and relapse-free survival (RFS; $P = .021$). Ferritin >685 ng/mL was associated with a higher incidence of relapse ($P = .005$) and relapse mortality ($P < .001$), but not of nonrelapse mortality (NRM; $P = .23$). Similar results were seen when pretransplantation ferritin was analyzed as a continuous variable and by quartiles. Our results indicate the need for studies designed to correlate an elevated ferritin with iron overload and to analyze the benefit of strategies to reduce the extent of iron overload.

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KEY WORDS: Iron overload, Pretransplantation ferritin, Autologous hematopoietic transplantation

INTRODUCTION

Iron overload is an adverse prognostic factor for patients undergoing allogeneic stem cell transplantation for thalassemia [1,2]. Elevated levels of serum ferritin, the most widely used surrogate for iron stores, are associated with worse outcomes following transplantation for acute leukemia and myelodysplastic syndrome (MDS) [3] and are also associated with an increased risk of organ toxicity following autologous stem cell transplantation (ASCT) [4,5]. The aim of this study was to determine the relationship between pretransplantation ferritin levels and outcome following ASCT for Hodgkin and non-Hodgkin lymphoma.

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METHODS

Patients

The records of 375 consecutive adult patients who underwent ASCT for Hodgkin (HL) and non-Hodgkin lymphoma (NHL) at the Cleveland Clinic between November 2000 and December 2006 were reviewed. A pretransplantation serum ferritin (drawn within 100 days preceding transplant admission) was available for 315 patients (86%) who form the patient population for this report.

Institutional review board approval was obtained to perform this study in accordance with the Declaration of Helsinki.

Peripheral Blood Stem Cell (PBSC) Mobilization and Autologous Transplantation

Two hundred sixty-four patients received etoposide 2 g/m^2 with granulotyce-colony stimulating factor (G-CSF) 10 μg/kg/day subcutaneously for mobilization of stem cells, 35 patients underwent mobilization with G-CSF alone, 13 patients with G-CSF with AMD-3100, and 3 patients with G-CSF and other chemotherapy agents. A minimum dose of 2.0×10^6 CD34$^+$ cells/kg was required to proceed with transplantation.

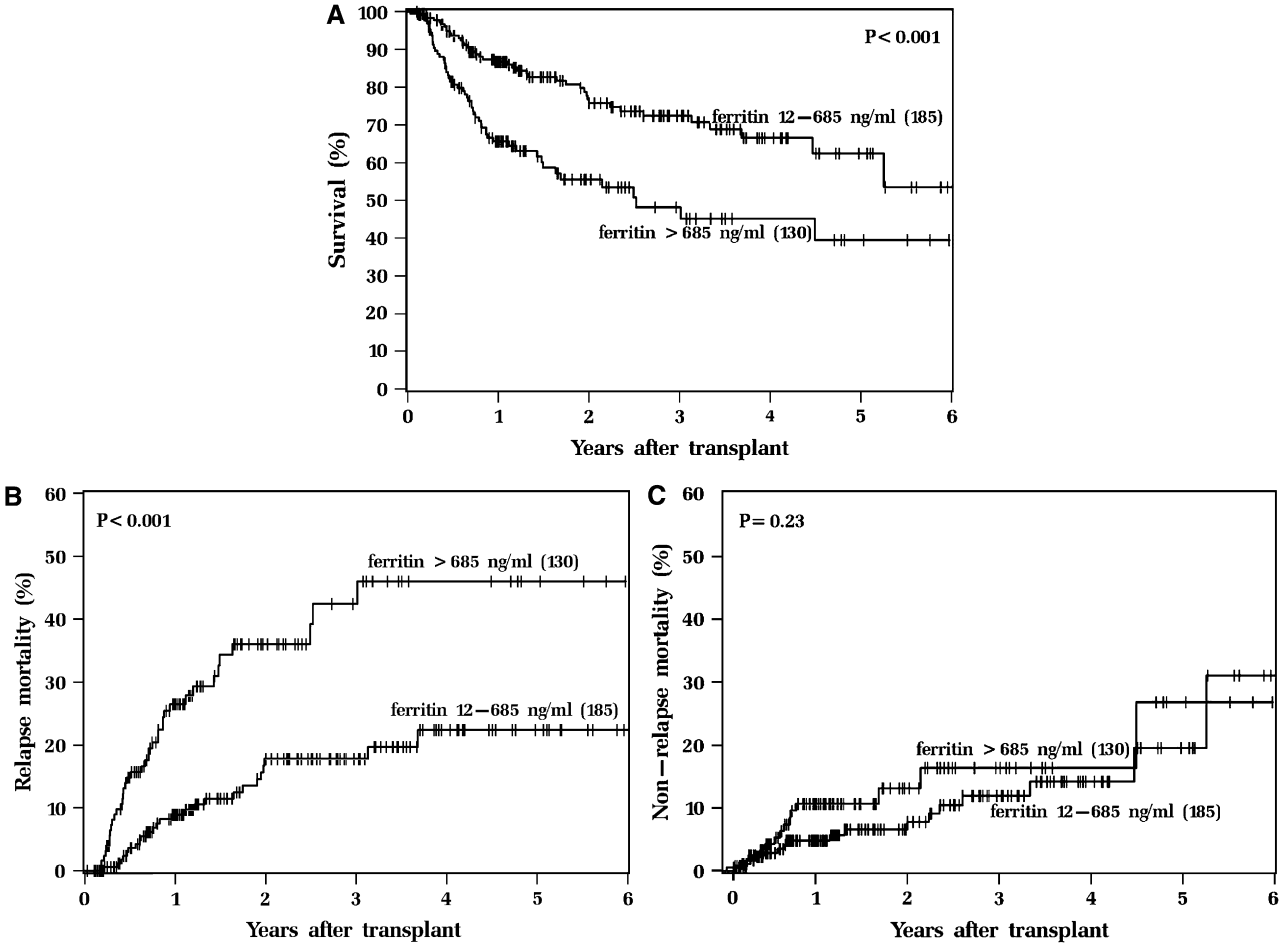

Figure 1. Outcome of patients stratified by pretransplantation ferritin level. Patients are stratified using recursive partitioning analysis. (A) overall survival. (B) relapse mortality. (C) non relapse mortality.

Three hundred twelve patients received a preparative regimen of oral busulfan (Bu) 14 mg/kg, etoposide 60 mg/kg, and cyclophosphamide (Cy) 120 mg/kg. Three patients received Bu 16 mg/kg and Cy 120 mg/kg.

Patients were hospitalized for administration of the preparative regimen and were routinely discharged after hematologic recovery.

Statistical Analysis

Recursive partitioning analysis (RPA) with a log-rank splitting method was used to identify the cut off point in baseline ferritin that best correlated with overall survival (OS) among the 315 study patients, regardless of other study variables. This cut off point was used in the analysis of all outcomes, as were ferritin as a continuous variable, and ferritin quartiles. The association of pretransplantation characteristics with elevated ferritin was assessed using the chi-square test (categoric variables) or Wilcoxon test (continuous variables). Outcomes were estimated using the Kaplan-Meyer method and compared between patients with and without elevated ferritin using the log-rank test. Outcomes included OS, relapse-free survival (RFS), relapse, relapse mortality, and nonrelapse mortality (NRM).

Cox proportional hazards analysis was used to identify univariable and multivariable risk factors for relapse, OS, and RFS. Because the focus of this study was to assess the prognostic effect of ferritin after adjusting for other study variables, each multivariable Cox model included ferritin and any factor prognostic for the outcome at $P < .10$. Each of these variables was retained in the multivariable model regardless of its statistical significance or lack thereof. The factors assessed include age at transplant, sex, primary diagnosis, months from diagnosis to transplantation, number of prior chemotherapy regimens, prior radiation therapy, ferritin, albumin, bilirubin, aspartate transaminase (AST), alkaline phosphatase, lactate dehydrogenase (LDH) pretransplantation, disease status at transplant, preparative regimen, and $CD34^+$ dose. Because most of the patients in the analysis were referred from and returned to outside practices after transplant, an accurate history of the number of red blood cell transfusions prior to or following transplantation was not possible.

Table 1. Patient Characteristics

	Ferritin ≤685 ng/mL (185)	Ferritin >685 ng/mL (130)	
	N (%)	N (%)	*P*-value
Median age at transplant, years (range)	51(19-77)	55(23-75)	.011
Sex, male	106(57)	94(72)	.006
Diagnosis			
Non-Hodgkin lymphoma	146(79)	111(85)	.14
Hodgkin lymphoma	39(21)	19(15)	
Median interval from diagnosis to transplant, months (range)	16(2-233)	19(4-372)	.19
<9 months	50(27)	27(21)	.58
9-18 months	50(27)	36(28)	
18-44 months	45(24)	33(25)	
>44 months	40(22)	34(26)	
Prior radiation therapy	56(31)	33(26)	.36
Courses of chemotherapy			
1	54(30)	12(9)	<.001
2	89(49)	66(52)	
3	28(15)	35(27)	
4-12	12(7)	15(12)	
Albumin <4.0	36(20)	31(25)	.29
Bilirubin ≤1×ULN	182(100)	123(99)	.22
AST ≤2×ULN	180(99)	123(99)	.80
Alkaline phosphatase >1×ULN	32(18)	51(41)	<.001
LDH >1×ULN	114(62)	80(63)	.85
Disease status at transplant			
Complete remission 1	13(7)	7(5)	.72
Complete remission 2+	34(19)	19(15)	
Partial remission	121(66)	91(71)	
Relapsed	15(8)	12(9)	
Preparative regimen			
Bu/Cy/VP-16	183(99)	129(99)	.78
Bu/Cy	2(1)	1(1)	
$CD34^+$ dose, $\times 10^6$/kg	8.98(2.01-65.18)	7.15(2.04-42.75)	<.001

AST indicates aspartate transaminase; LDH, lactate dehydrogenase; ULN, upper limit of normal.

Results of Cox proportional hazards analyses are summarized as the hazard ratio (HR) and 95% confidence interval (CI) for the hazard ratio. $P < .05$ was used to indicate statistical significance for all analyses. Analyses were performed using SAS® software (SAS Institute Inc., Cary, NC).

RESULTS

The survival of the 315 study patients did not differ significantly from that of the 60 patients without an available baseline ferritin ($P = .26$).

The normal range for ferritin in our laboratory is 18 to 300 ng/mL. The median pretransplantation ferritin for patients in this study was 577 ng/mL (range: 12.8-4115). RPA identified a baseline ferritin >685 ng/mL as the cut off point that best correlated with poor survival (Figure 1A); 130 patients (41%) had a pretransplantation ferritin >685 ng/mL. The clinical characteristics of the 130 patients with a pretransplantation ferritin >685 ng/mL are compared with those of the 185 patients with a pretransplantation ferritin ≤685 ng/mL in Table 1. The number of courses of chemotherapy received prior to transplant was significantly higher ($P < .001$) in patients with a pretransplantation ferritin >685 ng/mL. The median follow-up for surviving patients is 18.2 months (range: 1.2-75.4).

Univariable analysis demonstrated that age, number of prior chemotherapy regimens, low albumin, elevated alkaline phosphatase, and elevated ferritin were significantly associated with OS. The univariable analysis of prognostic factors assessed for influence on OS, RFS, and relapse is summarized in Table 2.

Multivariable analysis demonstrated that elevated ferritin ($P = .002$, HR 2.02, 95% CI 1.29-3.16) and low albumin ($P = .025$, HR 1.67, 95% CI 1.07-2.60) were significant adverse prognostic factors for OS. Elevated ferritin ($P = .021$, HR 1.60, 95% CI 1.08-2.38) and low albumin ($P = .008$, HR 1.70, 95% CI 1.15-2.51) were also adverse factors for RFS (Table 3).

Ferritin > 685 ng/mL was associated with a higher incidence of relapse ($P = .005$) and relapse mortality ($P < .001$; Figure 1B), but not of NRM ($P = .23$; Figure 1C).

When pretransplantation ferritin was analyzed as a continuous variable, it was significantly associated with OS ($P = .008$, HR 1.01, 95% CI 1.01-1.06), RFS ($P = .006$, HR 1.03, 95% CI 1.01-1.06), relapse ($P = .008$, HR 1.04, 95% CI 1.01-1.06), and relapse mortality ($P < .001$, HR 1.05, 95% CI 1.02-1.08), but not with NRM ($P = .65$, HR 1.01, 95% CI

Table 2. Prognostic Factors: Univariable Analysis

Variable	Overall Survival			Relapse-Free Survival			Relapse		
	HR	95%CI	*P*	HR	95%CI	*P*	HR	95%CI	*P*
Age at transplant, per 10-year increase	1.19	1.01-1.40	.034	1.08	0.94-1.24	.29	1.02	0.87-1.18	0.84
Male sex	1.19	0.78-1.83	.42	1.13	0.78-1.64	.51	1.34	0.87-2.05	0.18
Primary diagnosis HL/NHL	0.99	0.59-1.66	.98	1.08	0.69-1.68	.74	1.08	0.66-1.79	0.75
Months from diagnosis to transplant, per 6 month increase	1.00	0.98-1.03	.7	0.99	0.97-1.02	.63	0.98	0.95-1.01	0.20
9.01-18/<9	1.46	0.84-2.56	.18	1.23	0.77-1.98	.39	1.23	0.73-2.07	0.44
18.01-44/<9	0.89	0.47-1.71	.73	0.84	0.49-1.43	.52	0.85	0.48-1.52	0.59
>44/<9	1.32	0.73-2.37	.36	0.99	0.60-1.65	.98	0.80	0.44-1.43	0.45
Number of prior chemotherapy regimens, per 1 regimen increase	1.24	1.04-1.47	.016	1.16	1.00-1.34	.047	1.16	0.99-1.37	0.07
Prior radiation therapy	1.28	0.84-1.97	.25	1.02	0.69-1.50	.93	0.90	0.58-1.41	0.65
Disease status at transplant									
$CR2^+$/CR1	1.53	0.44-5.39	.50	1.70	0.58-5.03	.34	2.30	0.51-10.26	0.28
PR/CR1	1.86	0.58-5.91	.30	2.08	0.76-5.69	.15	3.40	0.83-13.86	0.09
Relapsed/CR1	2.71	0.74-9.86	.13	3.50	1.15-10.6	.028	6.54	1.48-29.04	0.014
$CD34^+$ dose, per 5 x 10^6/kg increase	0.89	0.77-1.03	.12	0.89	0.79-1.01	.06	0.90	0.79-1.03	0.14
Bu/Cy/VP preparative regimen	0.71	0.10-5.12	.74	1.05	0.15-7.50	.96	0.83	0.12-5.96	0.85
LDH >1xULN	1.38	0.90-2.12	.14	1.16	0.80-1.68	.43	1.20	0.79-1.83	0.38
Bilirubin, per 0.1 unit increase	1.02	0.92-1.14	.66	0.99	0.90-1.10	.91	0.97	0.86-1.09	0.60
Alkaline phosphatase > 1xULN	2.22	1.45-3.38	<.001	1.93	1.33-2.80	<.001	1.59	1.03-2.45	0.036
Aspartate transaminases, 2xULN	0.76	0.10-5.42	.78	0.64	0.09-4.56	.65	0.83	0.12-5.98	0.86
Albumin <4.0	1.88	1.22-2.90	.004	2.00	1.37-2.92	<.001	1.93	1.26-2.96	0.002
Ferritin >685	2.35	1.56-3.53	<.001	1.80	1.26-2.56	.001	1.76	1.18-2.62	0.005

HR indicates hazard ratio; CI, confidence interval; HL, Hodgkin lymphoma; NHL, non-Hodgkin lymphoma; CR, complete remission; ULN, upper limit of normal.

0.96-1.07). In addition, we examined pretransplantation ferritin in quartiles and found a linear association of ferritin with OS (Figure 2A) and relapse mortality (Figure 2B) but not NRM (Figure 2C).

DISCUSSION

Elevated pretransplantation ferritin levels are associated with increased mortality and worse transplant-related survival following allogeneic hematopoietic stem cell transplantation. Because of its predictive value, the pretransplant ferritin level has been incorporated into a prognostic scoring system for patients undergoing myeloablative allogeneic transplantation for acute leukemia and MDS [6].

This study demonstrates an association between elevated ferritin levels and OS and RFS following ASCT for lymphoma. Although the association of elevated ferritin with outcome is presumed to relate to its correlation with increased iron stores, ferritin may be elevated in other circumstances, including inflammation. Notably, the inclusion of albumin in the multivariate model, as a negative acute phase reactant [3], did not alter the impact of ferritin on outcome. Erythrocyte sedimentation rate or C-reactive protein (CRP) values, markers of acute inflammation, were not routinely performed, and hence were not included in the analysis. Elevated ferritin may also occur in association with abnormal liver function tests and greater extent of malignancy, but these parameters were accounted for in multivariate analysis.

Free iron can lead to oxygen-derived free radical-mediated tissue injury [7]. In patients undergoing bone marrow transplantation serum iron and ferritin rise 2 to 3 days prior to bone marrow infusion [8], and nontransferrin bound serum iron appears shortly after the start of conditioning and remains detectable in most patients throughout the peritransplantation period [9]. Previous studies have postulated that iron overload may increase susceptibility to organ damage and risk of infections [10-12]. Yet this study demonstrates no significant increase in NRM in patients with elevated ferritin levels. Improved supportive care, particularly management of infectious complications following autologous transplantation, may have overcome any potential adverse impact on NRM. In addition, even though our study cohort is large, the incidence of NRM is low and a statistically significant difference would be difficult to detect.

Unexpectedly, the adverse influence of elevated ferritin occurs predominantly through its association with relapse and relapse mortality. Iron plays a critical role in cell proliferation; tumor cells require more iron for DNA synthesis than normal cells probably related to their rapid proliferation [13]. Decreased tumor growth has been observed in iron-deficient mice [14]. Furthermore, iron chelators inhibit tumor cell growth in vitro [15]. Thus, accumulating data suggests that elevated iron stores, apart from providing a milieu for infection and organ toxicity and hence increased risk for

Table 3. Prognostic Factors: Multivariable Analysis

Variable	HR	95% CI	*P*
Multivariable model for overall survival			
Ferritin >685 ng/mL	2.02	1.29-3.16	.002
Albumin <4 g/dL	1.67	1.07-2.60	.025
Multivariable model for relapse-free survival			
Ferritin >685 ng/mL	1.60	1.08-2.38	.021
Albumin <4 g/dL	1.70	1.15-2.51	.008
Multivariable model for relapse			
Ferritin >685 ng/mL	1.51	0.97-2.40	.049
Albumin <4 g/dL	1.64	1.06-2.55	.026
Relapsed disease at time of transplant	4.78	1.06-21.64	.042

HR indicates hazard ratio; CI, confidence interval.

NRM, may also influence tumor growth. Hopefully, our demonstration of an association of ferritin level with relapse and relapse mortality will add to the impetus for further study. It is an intriguing finding, but requires prospective validation.

It is important to recognize the limitations of this study. Ferritin is used here and in other reports as an indicator of iron overload, but its correlation with iron overload is imprecise. Ferritin may be a surrogate for more advanced disease and thus have an impact on relapse. As in other studies [16], the incorporation of disease status at the time of transplantation and interval from diagnosis to transplantation in multivariate analysis does not exclude a potential effect of the malignancy on ferritin levels. Studies of some nonhematologic malignancies suggest an adverse prognostic impact of elevated ferritin even after adjustment for tumor and patient characteristics [17,18]. The present study cannot distinguish between a causal relationship of elevated ferritin, through iron stores, on the malignancy and ferritin being a surrogate for extent or aggressiveness of malignancy.

Magnetic resonance imaging (MRI) assessment of iron overload in the heart and liver of hematopoietic stem cell transplant recipients reveals correlation of hepatic T2* abnormalities with abnormal ferritin levels [19]. A recent study reporting the prevalence of iron overload following allogeneic transplantation indicates that, although serum ferritin is a good screening test, the liver iron concentration using R2 MRI correlates only moderately with serum ferritin [20]. It will be important to prospectively estimate the prevalence of clinically significant iron overload in patients

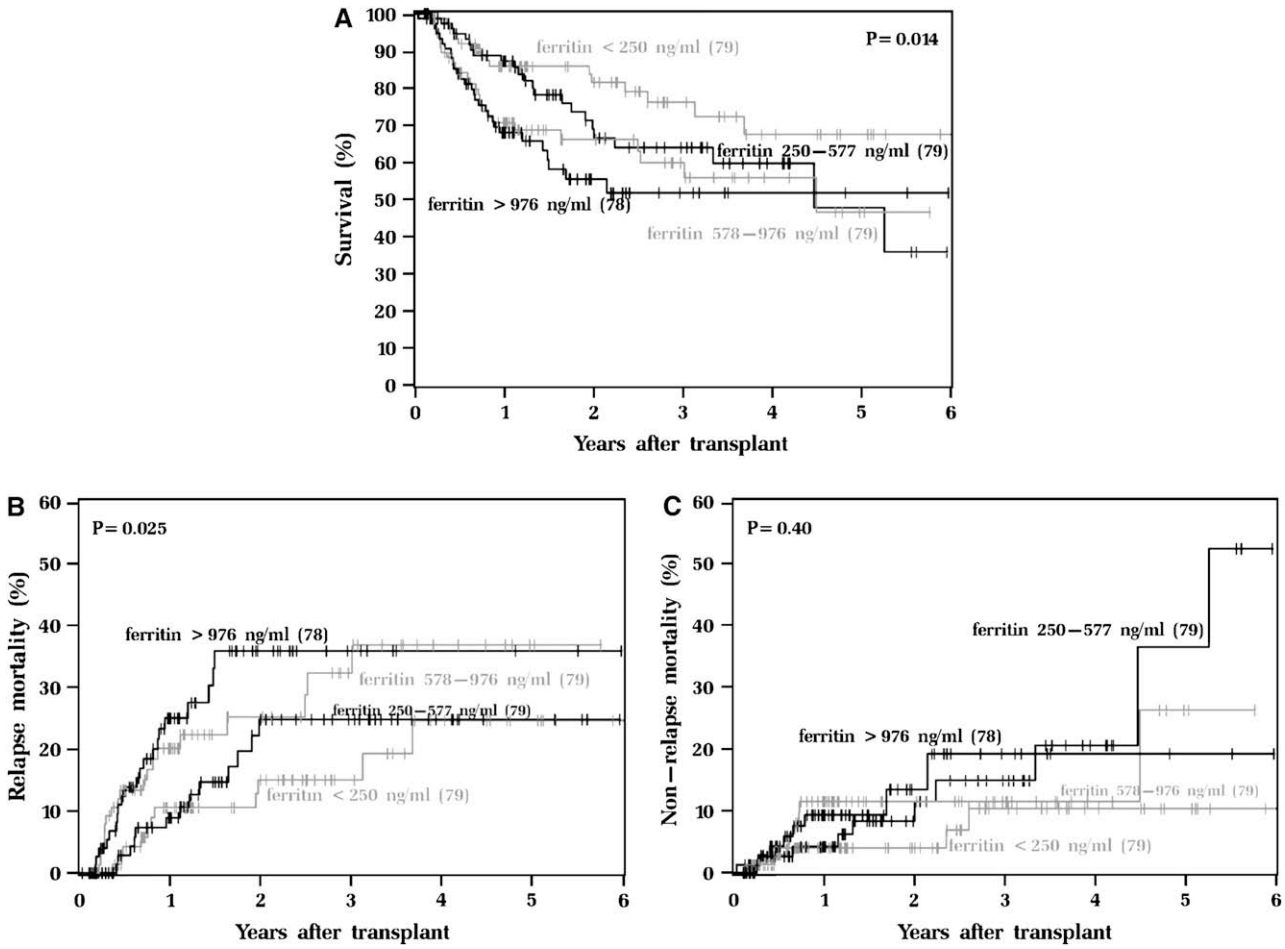

Figure 2. Outcome of patients stratified by pretransplantation ferritin level. Patients are stratified using quartiles. (A) overall survival. (B) relapse mortality. (C) non relapse mortality.

undergoing ASCT with techniques such as liver-specific MRI and to analyze the correlation with serum ferritin levels [19-21]. This approach might provide insight into the mechanisms by which elevated ferritin influences transplant outcome and would better determine whether patients might benefit from interventions to reduce iron overload.

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